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Controlled-release in-situ gel forming formulation of tramadol containing chitosan-based pro-nanogels

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ABSTRACT

Chronic pain is one of the most prevalent health problems worldwide. Tramadol is a synthetic semi-opioid analgesic, interacting with serotonergic, adrenergic and opioid receptors to reduce the pain but its short half-life in vivo may reduce patient compliance in case of chronic pains. To overcome this problem, novel drug delivery systems have been investigated. This study focuses on a chitosan based thermoresponsive in-situ gel forming formulation intended to subcutaneous injection. To evaluate further drug release, a reversed phase high performance liquid chromatography method was developed. Then two formulations (with and without TPP) were optimized by D-optimal plan using Design-Expert statistical software and were characterized in terms of morphology, release phenomenon, texture, swelling and stability as well as in vivo response. AFM images show approximately spherical nanocavities in the homogenous TPP containing gel structure, which explain the different patterns of drug release between the two formulations. This implies that changing TPP concentration can control formation of these cavities and hence drug release rate and kinetics. Not present in the sol state, nanostructures lead to emerge of a new concept: pro-nanogels. Finally, the formulations with proper texture qualities, stability and rapid sol-gel transition in vivo could be a candidate for controlled release of therapeutic agents following subcutaneous injection.

1. Introduction

Chronic pain is one of the most prevalent and burdensome health problems worldwide and causes billions of dollars cost for health care systems besides the indirect costs of less productivity in working population [1]. It is estimated to afflict more individuals as the population ages and reduces the quality of life of elderlies. A noticeable meta-analysis in 2016 reveals that approximately one third to a half of UK population suffers chronic pain with various etiologies and severities [2]. In 2014, it is also stated that almost 100 million people in the US experience the problem [3]. Pharmacological interventions for chronic pain include prescribing acetaminophen, NSAIDs, COX II inhibitors and opioids. Opiates may only prescribe when other mild medications have not been successful in pain management or their adverse effects are unbearable [4].

With the minimum risk of abuse among opioids, tramadol is a synthetic semi-opioid analgesic agent with an active metabolite, *O*-desmethyl tramadol, interacting with serotonergic, adrenergic and opioid receptors to reduce the pain [5, 6]. Its in vivo half-life is about 6 h and is mainly excreted by kidney. As Tramadol half-life necessitates its use every 4–6 h, which is inconvenient in chronic pain, it is required that extended and long acting dosage forms of tramadol introduce to market [7–9]. Several extended release tablets and capsules of tramadol in different doses have been recently marketed in North America [10]. Besides, academic attempts are still done to introduce more effective controlled release formulations. Microsphere carriers for epidural injection and biodegradable implants based on synthetic or natural polymers have been widely studied. It is reported that a chitosan based implant has been successful to extend tramadol release up to 17 days [11]. Also an in-situ sol-gel transitory system based on poloxamer hydrogel has been reported to be successful in sub-cutaneous injection for tramadol administration [12].

Hydrogels are three dimensional networks of cross-linked hydrophilic polymers swelling in water or biologic fluids [13]. Because of hydrogels' good biocompatibility, solute permeability and acceptable release characteristics, they have been utilized as drug carriers, protein, cells and others. In-situ forming gels show sol-to-gel transition in response to one or a combination of two or more stimuli including UV-irradiation, temperature and pH change, solvent exchange, etc. at the in-situ site, where they are administrated into the body [14]. Thermoresponsive gel forming polymers can form a type of in-situ forming gels whose sol-gel transition happens upon an alteration in

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Table 1
Applied mathematical models for drug release kinetics study.

Model	Equation
Zero order equation	$F = k_0 \cdot t$
First order equation	$\ln(1 - F) = -k_1 t$
Higuchi's equation	$F = k_H \cdot t^{1/2}$
Hixson–Crowell model	$1 - (1 - F)^{1/3} = -k_{HC} \cdot t$
Square root of mass	$1 - (1 - F)^{1/2} = K_{SR} \cdot t$
Three second root of mass	$-(1 - F)^{2/3} = K_{TSR} \cdot t$
Weibull equation	$\ln[-\ln(1 - F)] = \beta \ln t_d + \beta \ln t$
Korsmeyer–Peppas	$\ln F = \ln k_{kp} + n \ln t$

temperature. In these systems, the drug can be mixed with the aqueous sol for convenient administration and then a gel encapsulating drug is formed in-situ.

Chitosan is a natural polysaccharide that is widely used for biomedical applications because of its good biocompatibility, biodegradability, nontoxicity, and low immunogenicity. Chitosan-based in-situ gelling systems are recognized as smart biomaterials for biomedical applications such as drug delivery. These systems can be delivered in minimally invasive techniques such as injection, ocular or nasal administration while protecting drugs from the hostile environment could be another advantage for them [15].

In the present study our goal is preparation, optimization and characterization of a parenteral chitosan-based in-situ gel forming formulation of tramadol intended to use in chronic pains.

2. Materials and method

2.1. Materials

Intermediate molecular weight chitosan (Sigma Aldrich, USA), pentasodium triphosphate (TPP) (Merck, Darmstadt, Germany), tramadol hydrochloride (kindly donated by Alborz Darou, Iran), poloxamer F-127 (Sigma Aldrich, USA), acetic acid glacial (Merck, Germany), and glycerophosphate disodium salt hydrate (Sigma Aldrich, USA), were utilized to prepare formulations.

2.2. Drug assay

To evaluate further drug release from formulations, a reversed phase high performance liquid chromatography (RP-HPLC) was developed. A C18 column (C18, 250 × 4.6 mm, MZ Analysentechnik, Germany) as stationary phase, a mixture of acetonitrile, phosphate buffer (pH of 5.9, composed of KH_2PO_4 1.36 g and purified water q.s. 1000 ml) in 70:30 ratio and 0.1% tri-ethanol amine as mobile phase, a gradient pump controller unit (EA4300, Smartline pump 1000, Knauer, Germany) set to generate a 1 ml/min flow, while it was equipped with a Rheodyne injector and a Rheodyne 100 μl loop, and a UV detector (E4310, UV detector 2500, Knauer, Germany) set to detect tramadol at $\lambda = 218$ nm form the HPLC set-up.

2.3. Optimization

Chitosan, Glycerophosphate and poloxamer concentrations were optimized in regards to the least gelling time of the formulation with a D-optimal plan using Design-Expert statistical software (version 6.1, Stat-Ease Inc., USA). As the pH for the optimized formulation was around 5.4, to adjust the pH in physiologic range, 5, 10 and 15 μl of NaOH 10 N added to samples of optimized formulation; then exact pH and gelling time was evaluated for each sample. The effect of temperature on gelling time was also studied by estimating that in 25, 30, 32, 35 and 37 °C for samples of optimized formulation in each point. pH and temperature conditions were evaluated in triplicate for each point.

2.4. Preparation of in-situ gel forming formulation loaded by tramadol

To prepare the optimized in-situ gel forming formulation, chitosan (1%w/v) was dispersed in 0.1 N acetic acid solution. Then tramadol (20%w/w) and poloxamer F-127 (20%w/v) were dissolved in chitosan dispersion while stirring. After that, the whole mixture was transferred to ice bath for about 30 min and glycerophosphate disodium salt hydrate (14.5%w/v) and pentasodium triphosphate (0.5%w/v) were added drop-wisely to the chitosan mixture under stirring in the ice bath for about 1 h. Finally, the mixture transferred to 37 °C water bath and gel formation was obvious after about 1–1.5 min.

2.5. Formulation characterization

2.5.1. Morphology

The morphology of the optimized formulation in gel form was characterized using atomic force microscopy (AFM-JPK, NanoWizard® II, Germany). For AFM imaging, gel samples were fixed on a lamella, dried at room temperature and examined using AFM without being stained.

2.5.2. Drug release and kinetic studies

In vitro drug release profiles of in-situ gel forming formulation were done using Franz cells as the donor and receiver phase containers, separated by a dialysis membrane (cut-off 12 kDa, Art No. D9652, Dialysis Tubing Cellulose Membrane, Sigma Aldrich, USA). The cells were double-jacketed with 37 °C water circulation between the jacket walls. The receiver phase was consist of 23 ml phosphate buffered saline (PBS; pH of 7.4, disodium hydrogen phosphate 1.38 g, potassium dihydrogen phosphate 0.19 g, sodium chloride 8.0 g, purified water q.s. 1000 ml) and the donor phase was 70 mg of in-situ gel forming formulation (considering the sink condition) or equal dose of free drug solution. Finally the amount of tramadol in the receiver medium was analyzed in $t = 0, 0.25, 0.5, 1, 2, 4$, and 8 h using the developed HPLC method. All of the steps were repeated in triplicate.

To find out the best model describing the tramadol release from gel formulations, the release data were fitted to eight conventional models including zero order, first order, Higuchi, Hixson–Crowell, square root of mass, three second root of mass, Weibull and Korsmeyer–Peppas (Table 1). Then the accuracy and predictability of the suggested models were evaluated by calculation of coefficients of determination (R^2), sum

Table 2
Experimental design and results of D-optimal method.

Run	Independent variables			Dependent variables
	Chitosan conc.%	Poloxamer conc.%	Glycerophosphate conc.%	Gelling time (s)
1	1.5	15	40	900
2	1.5	25	22.9	60
3	0.5	25	40	69
4	0.5	15	10	Not formed
5	1.5	23	40	35
6	1.5	18	10	2041
7	0.5	15	40	Not formed
8	1.1	25	10	68
9	0.5	22.9	10	75
10	1	19.1	26.2	1413
11	1	21.4	40	71
12	0.6	23	25.6	67
13	1	15	13.8	Not formed
14	1.5	17	25.5	686
15	0.5	15.8	25	Not formed
16	1.5	18	10	2419
17	0.5	25	40	58
18	1.5	15	40	Not formed
19	0.5	15	40	Not formed
20	1.1	25	10	3603

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